PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 482119 CJE/fjw	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/NZ2003/000109**	International Filing Date *(day/month/year)* 30 May 2003	Priority Date *(day/month/year)* 30 May 2002
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] C12N 15/12, A61K 38/16, A61K 39/395, A61P 15/00		
Applicant DAVIS, George Henry et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of **14** sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand 24 December 2003	Date of completion of the report 16 September 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **ALISTAIR BESTOW** Telephone No. (02) 6283 2450

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ2003/000109

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1 – 14, 18 – 26, 28, 29, 31, 33 - 48, 50 - 68** as originally filed,
pages **15, 16, 49** received on **30 July 2004** with the letter of **27 July 2004**,
pages **17, 27, 30, 32** received on **10 September 2004** with the letter of **10 September 2004**

☒ the claims, pages , as originally filed,
pages , filed with the demand
pages **69, 70** received on **30 July 2004** with the letter of **27 July 2004**
pages **71 - 75** received on **10 September 2004** with the letter of **10 September 2004**

☒ the drawings, pages **1/13 – 13/13** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the sequence listing part of the description:
pages **1 - 30** as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

☒ The amendments have resulted in the cancellation of:

☐ the description, pages

☒ the claims, page no. **76**

☐ the drawings, sheets/fig.

☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ2003/000109

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1 - 39	YES
	Claims	NO
Inventive step (IS)	Claims 1 – 39	YES
	Claims	NO
Industrial applicability (IA)	Claims 1 - 39	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

Citations

D1 WO 2001/085926 A2 (AGRESEARCH LIMITED) 15 November 2001.
D2 WO 2001/096393 A2 (AGRESEARCH LIMITED) 20 December 2001.
D3 Juengel et al (2002) Biology of Reproduction 67: 1777–1789.
D4 WO 1999/017797 (THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE) 15 April 1999.
D5 Vitt et al (2000) Endocrinology 141: 3814–3820.
D6. WO 2000/066620 (CREATIVE BIOMOLECULES INC) 9 November 2000.
D7 Yan et al (2001) Molecular Endocrinology 15: 854–866.
D8 Montgomery et al (2001) Reproduction 121: 843–852.

Novelty (N) and Inventive Step (IS)

D1 claims a method of increasing (claim 17), or modulating (claim 16) the ovulation rate, or inducing sterility (claim 19) in a female mammal, by administering an effective amount of particular GDF-9B polypeptides being selected from SEQ ID 2,4,6 or 8.

D3 discloses the immunisation of sheep with GDF-9 and GDF-9B (BMP-15).

D4 discloses a method of inhibiting oocyte maturation by contacting the oocytes with GDF-9 (claim 8).

D5 discloses treatment of female rats with recombinant GDF-9 stimulated follicle development. This has an effect on ovulation.

D6 discloses the administration of morphogens, including GD-9 and GDF-9A (BMP-15) as luteinizing inhibitors or modulators of the corpus luteum (claim 15).

While the present application does refer to modulating the ovulation rate and immunising sheep, it does so by use of sequences other than those used in D1, D3, D4, D5 and D6.

The methods and sequences the subject of the claims would not be obvious from any one, or any combination of D1 – D8, and therefore the claimed matter appears to be inventive.

Industrial Applicability (IA)

Claims 1 - 39 meet the requirements of the PCT with regard to industrial applicability.

The present invention also provides a method of identifying a mammal which carries a mutated nucleic acid molecule encoding GDF-9B, said method comprising the steps of:

i) obtaining a tissue or blood sample from the mammal;

ii) isolating DNA from the sample; and optionally

iii) isolating GDF-9B DNA from the DNA obtained at step i) or ii);

iv) probing said DNA with a probe complementary to either strand of the mutated GDF-9B DNA of SEQ ID NOs 11 or 17;

v) amplifying the amount of mutated GDF-9B DNA;

vi) determining whether the GDF-9B sequence DNA obtained in step v) carries a mutation associated with sterility or increased ovulation; and

vii) optionally selecting a mammal which carries a mutation associated with increased ovulation for breeding.

The present invention further provides a method of identifying a mammal which carries a mutated nucleic acid molecule encoding GDF-9, said method comprising the steps of:

i) obtaining a tissue or blood sample from the mammal;

ii) isolating DNA from the sample; and optionally

iii) isolating GDF-9 DNA from the DNA obtained at step i) or ii);

iv) probing said DNA with a probe complementary to either strand of the mutated GDF-9 DNA of SEQ ID NO 5;

v) amplifying the amount of mutated GDF-9 DNA;

vi) determining whether the GDF-9 sequence DNA obtained in step v) carries a mutation associated with sterility or increased ovulation; and

vii) optionally selecting a mammal which carries a mutation associated with increased ovulation for breeding.

Alternatively, said antibodies may be administered directly in a partial or short term passive immunisation regime.

A decrease in ovulation rate sufficient to reduce fertility or induce sterility may be induced by mimicking the homozygous state, whereby no active GDF-9 and/or GDF-9B is expressed. This can be achieved by a full or long term active immunisation regime whereby wild-type GDF-9 and/or GDF-9B or a functional variant or fragment thereof is administered to raise sufficient antibodies to affectively neutralise all of the endogenous GDF-9 and/or GDF-9B. Alternatively, said antibodies may be administered directly in a full or long term passive immunisation regime. Where the effect is permanent, sterility in the mammal is induced. Where the effect is reversible or temporary, a contraceptive effect is induced.

Thus in a further aspect, the present invention provides a method altering GDF-9 and/or GDF-9B bioactivity in a female mammal so as to modulate ovulation comprising the steps of either:

(a) inducing a partial immunisation response to endogenous GDF-9 and/or GDF-9B to partially reduce bioactivity thereof and enhance ovulation; or

(b) inducing a full immunisation response to endogenous GDF-9B or GDF-9 and GDF-9B together to substantially reduce bioactivity thereof and induce sterility;

wherein the immunisation response is induced by administration of an antigenic composition comprising:

i) a GDF-9 polypeptide or a functional fragment or variant of GDF9; and/or

ii) a GDF-9B polypeptide selected from SEQ ID NOs: 8, 10, 12, 14, 16 and 18;

administered in sufficient amounts, may compete with the endogenous GDF-9 and/or GDF-9B binding and/or dimerisation to reduce the biological activity thereof. Such a response, if it results in a partial reduction of endogenous GDF-9 and/or GDF-9B activity will result in enhanced ovulation and fertility. If such administration results in a full reduction of endogenous GDF-9 and/or GDF-9B activity, the response induced will be sterility.

Also disclosed is a transgenic non-human mammal wherein one copy of the endogenous GDF-9 and/or GDF-9B gene has been knocked out. Such a mammal would have increased ovulation and enhanced fertility.

Such a transgenic mammal may be produced by known methods (Wells et al, 1998. Reprod Fertil Dev: 10:615-26; Clark 2002, Methods Mol Biol, 180: 273-87; Cousens et al 1994, Mol Reprod Dev. 39:384-91; Chen et al 2002, Biol Reprod. 67: 1488-92; Arat et al 2001, Mol Repord Dev. 60: 20-6) and may comprise the steps of introducing to the genetic material of the mammal at least one nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- SEQ ID NOs 1 or a functional fragment or variant thereof; and
- SEQ ID NOs 7 or 13 but not both; or
- SEQ ID NOs 3 or a functional fragment or variant thereof; and
- SEQ ID NOs 9 or 15 but not both, using a vector or construct according to the invention.

In a further aspect the present invention provides a method of modulating the ovulation rate of a female mammal comprising the steps of:

A) SEQ ID NO 5; or

B) a functional variant or fragment of the molecule in A); or

C) a sequence complementary to the molecule in A) or B); and/or

ii) a single copy of mutated GDF-9B nucleotide sequence comprising:

A) SEQ ID NOs 11 or 17; or

B) a sequence complementary to the molecule(s) in A).

The mammals selected for breeding according to the method described above may result in progeny having the following characteristics:

i) a single copy of a mutated GDF-9 nucleotide sequence comprising:

A) SEQ ID NO 5; or

B) a functional variant or fragment of the molecule in A); or

C) a sequence complementary to the molecule in A) or B);

ii) a single copy of a mutated GDF-9B nucleotide sequence comprising:

A) SEQ ID NOs 11 or 17; or

B) a sequence complementary to the molecule(s) in A).

Also described is a method for selecting a female mammal for breeding on the basis of possessing an increased rate of ovulation comprising the steps of identifying a female mammal possessing only a single mutated copy of:

vi) determining whether the GDF-9B sequence DNA obtained in step v) carries a mutation associated with sterility or increased ovulation; and

vii) optionally selecting a mammal which carries a mutation associated with increased ovulation for breeding.

12. A method of identifying a mammal which carries a mutated nucleic acid molecule encoding GDF-9, said method comprising the steps of:

i) obtaining a tissue or blood sample from the mammal;

ii) isolating DNA from the sample; and optionally

iii) isolating GDF-9 DNA from the DNA obtained at step i) or ii);

iv) probing said DNA with a probe complementary to either strand of the mutated GDF-9 DNA of SEQ ID NO 5;

v) amplifying the amount of mutated GDF-9 DNA;

vi) determining whether the GDF-9 sequence DNA obtained in step v) carries a mutation associated with sterility or increased ovulation; and

vii) optionally selecting a mammal which carries a mutation associated with increased ovulation for breeding.

13. A method as claimed in claim 11 or 12 wherein the mammal selected has both a single mutated copy of GDF-9 and GDF-9B.

14. A use of a nucleic acid molecule which is complementary to either strand of the mutated DNA of SEQ ID NOs. 11 or 17 as a marker to identify a mammal carrying a mutated nucleic acid molecule encoding GDF-9B.

15. A use of a marker as defined in claim 14 in a method for marker assisted selection of a mammal which possesses a genotype which is associated with either enhanced ovulation or sterility.

16. A use of a nucleic acid molecule which is complementary to either strand of the mutated DNA of SEQ ID NO 5 as a marker to identify a mammal carrying a mutated nucleic acid molecule encoding GDF-9.

17. A use of a marker as defined in claim 16, in a method for marker assisted selection of a mammal which possesses a genotype which is associated with either enhanced ovulation or sterility

18. A probe capable of specifically hybridising to either strand of the mutated GDF-9B DNA of SEQ ID NOs 11 or 17 under stringent hybridisation conditions.

19. A probe capable of hybridising to either strand of the mutated GDF-9 DNA of SEQ ID NO 5 under stringent hybridisation conditions.

20. A construct comprising a nucleic acid molecule as claimed in claim 1 or 2.

21. A vector comprising a nucleic acid molecule as claimed in claim 1 or 2.

22. A host cell which comprises a construct or vector as claimed in claim 20 or 21 which has been introduced therein.

23. A cell line comprising a host cell as claimed in claim 22.

24. A method of altering GDF-9 and/or GDF-9B bioactivity in a female mammal so as to modulate ovulation comprising the steps of either:

(a) inducing a partial immunisation response to endogenous GDF-9 and/or GDF-9B to partially reduce bioactivity thereof and enhance ovulation; or

(b) inducing a full immunisation response to endogenous GDF-9B or GDF-9 and GDF-9B together, to substantially reduce bioactivity thereof and induce sterility;

wherein said immunisation response is induced by administration of an antigenic composition comprising:

i) a GDF-9 polypeptide or a functional fragment or variant of GDF9; and/or

ii) a GDF-9B polypeptide selected from SEQ ID Nos: 8, 10, 12, 14, 16 and 18;

together with a pharmaceutically or veterinarily acceptable carrier and/or diluent;

to a mammal in need thereof.

25. A method as claimed in claim 24, wherein said antigenic composition comprises a mild adjuvant to induce a partial immunisation response and induce enhanced ovulation.

26. A method as claimed in claim 24, wherein said antigenic composition comprises a strong adjuvant to induce a full immunization response and induce sterility.

27. A method as claimed in any one of claims 23 to 26, wherein said partial immunization response is induced by a short term immunization regime.

28. A method as claimed in any one of claims 23 to 26, wherein said full immunization response is induced by a long term immunization regime.

29. A method as claimed in claim 24, wherein said immunization response is induced passively by administration of antibodies raised against said antigenic composition.

30. A method as claimed in claim 29, wherein said antibodies are administered according to a short term regime to induce a partial immunization response and induce enhanced ovulation.

31. A method as claimed in claim 29, wherein said antibodies are administered according to a long term regime to induce a full immunization response and induce sterility.

32. A method as claimed in any one of claims 24, 26, 28, 29, and 31, wherein said full immunization response is temporary and/or reversible and wherein said sterility induced comprises contraception.

33. A method as claimed in any one of claims 24, 26, 28, 29, and 31, wherein said full immunization response and said sterility induced is permanent.

34. A method for breeding a mammal having increased ovulation comprising the steps of:

a) identifying the nucleotide sequences of GDF-9 or GDF-9B carried by the female mammal it is proposed to breed from;

b) identifying the nucleotide sequences of GDF-9 or GDF-9B carried by the male mammal it is proposed to breed from;

c) selecting the female and male animals that will result in progeny having the following characteristics:

i) a single copy of a mutated GDF-9 nucleotide sequence comprising:

A) SEQ ID NO 5; or

B) a functional variant or fragment of the molecule in A); or

C) a sequence complementary to the molecule in A) or B); and/or

ii) a single copy of mutated GDF-9B nucleotide sequence comprising:

A) SEQ ID NOs 11 or 17; or

B) a sequence complementary to the molecule(s) in A).

35. A method as claimed in claim 34, wherein said mammal is selected to have a single mutated copy of GDF-9 and GDF-9B.

36. A method of modifying the function of the corpus luteum by adminstering supplemnetary GDF-9 and/or GDF9-B to female mammals, wherein said GDF-9B is selected from the polypeptides of SEQ ID Nos: 8, 10, 12, 14, 16 and 18.

37. An isolated mutated GDF-9 polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, or 6 or a functional fragment or variant thereof.

38. An isolated mutated GDF-9B polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, 12, 14, 16, or 18.

39. A composition comprising an isolated nucleic acid as claimed in any one of claims 1 to 10, or an isolated polypeptide as claimed in claim 41 or 42 and a pharmaceutically acceptable carrier.

(Galloway *et al.*, 2000). Inactivating mutations in GDF-9B cause increased ovulation rate and infertility in a dosage dependent manner. The serine to isoleucine change in the [S2] mutation and premature stop codon in the [S1] mutation, support the notion that perturbations of protein structure within the GDF-9B mature peptide have serious consequences in protein activity.

Surprisingly, ewes which are heterozygous for both of the GDF-9 [787] mutation and one of the GDF-9B [S1] or [S2] mutations have an even higher ovulation rate than animals that are heterozygous for a GDF-9 or GDF-9B mutation alone. The effects of the combination of GDF-9 [787] and GDF-9B [S1] mutations in Cambridge sheep and the combination of GDF-9 [787] and GDF-9B [S2] mutation in Belclare sheep appears to be additive (Table 3).

Thus, it is contemplated that an immunisation regime which could mimic these genotypes would be useful in modulating ovulation in female mammals. For example, a regime which would reduce the activity of endogenous GDF-9B and/or GDF-9 to about one half (as in heterozygous animals whereby only 50% of active molecules are expressed) could be used to increase ovulation and enhance fertility in female mammals. Conversely, an immunisation regime which would reduce the activity of endogenous GDF-9 and/or GDF-9B to approximately zero (as in homozygous animals where no active molecules are expressed) could be used to induce sterility.

The present invention further provides an isolated GDF-9 nucleic acid molecule comprising at least one mutation at position 260, 471, 477, 721, 978, 994, 1111 or 1184 of the sequence. The mutation preferably results in an amino acid substitution in the polypeptide encoded by the nucleic acid molecule, and said amino acid substitution is preferably present in the receptor binding domain and causes a disruption in receptor

binding. Alternatively, the amino acid substitution may be present in the dimersation domain to cause a disruption in dimerisation.

The invention further provides an isolated GDF-9B nucleic acid molecule comprising at least one mutation at position 718, 747 or 1100 of the sequence. The mutation preferably results in an amino acid substitution in the polypeptide encoded by the nucleic acid molecule, and said amino acid substitution is preferably present in the receptor binding domain and causes a disruption in receptor binding. Alternatively, the amino acid substitution may be present in the dimerisation domain to cause disruption in dimerisation.

Suitable programs for ascertaining the structure of polypeptides from the amino acid sequence which can used to determine the regions of the nucleotide sequence associated with dimerisation and/or receptor binding will be known to persons skilled in the art. Examples of suitable computer programs include The Modeller by Rockerfeller University and The SWISS Model developed by Swiss Protein database.

The mutations seen in the GDF-9 and GDF-9B genes which are associated with changes in fertility in the Cambridge and Belclare breeds have been shown to be associated with alterations in the function of the encoded polypeptides due to amino acid substitutions. Comparison between the location of these amino acid substitutions, with mutations, in other closely related TGF-β molecules support the hypothesis that the biological activity of GDF-9 [787] is abolished due to a disruption in dimerisation whilst the GDF-9B [S2] mutation may abolish biological activity by disrupting receptor binding.

It is anticipated that other amino acid changes in the receptor-binding and dimerisation domains, or regions of the protein that disrupt protein folding of the mature peptide will have similar effects as would be appreciated by a skilled person and are included within the scope of the present invention.

breeding season (as assessed by lack of estrous activity in non-experimental sheep). Blood samples were collected from the ewes at 5 minutes, 1 h and 96 h after injection of the antiplasma and thereafter 3 times a week from the 2nd injection of Estrumate for determination of antibody titers and concentrations of progesterone in plasma.

Determination of progesterone concentrations

Concentrations of progesterone in plasma were determined by radioimmunoassay (RIA). The inter- and intra-assay co-efficients of variation were <10% and assay sensitivity was 0.1 ng/ml. All samples below the sensitivity of the assay were assigned a value of 0.1 ng/ml for statistical analysis.

Short-term immunisations

Romney ewes were immunized with KLH (N=50), KLH conjugated to GDF-9 peptide (N=30) or KLH conjugated to GDF-9B peptide (N=30). The antigens were administered in DEAE Dextran (5% w/v) on 2 occasions one month apart. The number of corpora lutea (CL) was determined following the first observed oestrus which occurred after the booster immunization. In addition, in a selected subpopulation of these ewes (N=26 KLH, N=15 GDF-9, N=16 GDF-9B) the number of CL present following the next oestrus was also determined. The average number of CL for each ewe was analysed by Chi-square analysis.

Statistical analysis

For the long-term, actively immunized ewes, ovulation rate for individual ewes was calculated as the mean of the number of corpora lutea observed at all observations for that ewe when at least 1 corpus luteum (CL) was present (i.e. observations of no CL were excluded from the calculation). The Kruskal-Wallis test was used to compare ovulation rates between the KLH-GDF-9B mature protein and the KLH treated groups.

WHAT WE CLAIM IS:

1. An isolated mutated GDF-9 nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

a) SEQ ID NOs. 1, 3 or 5;

b) a sequence complementary to the molecule defined in a);

c) a functional fragment or variant of the sequences in a) or b);

d) an anti-sense sequence to any of the molecules defined in a), b) or c).

2. An isolated mutated GDF-9B nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

a) SEQ ID NOs. 7, 9, 11, 13, 15 or 17;

b) a sequence complementary to the molecule defined in a)

c) an anti-sense sequence to any of the molecules defined in a) or b).

3. An isolated GDF-9 nucleic acid molecule comprising at least one mutation at position 260, 471, 477, 721, 978, 994, 1111 or 1184 of the nucleotide sequence.

4. An isolated GDF-9 nucleic acid molecule as claimed in claim 3, wherein said mutation results in an amino acid substitution in the polypeptide encoded by said nucleic acid sequence.

5. An isolated GDF-9 nucleic acid molecule as claimed in claim 4, wherein said amino acid substitution is present in a receptor binding domain and disrupts receptor binding.

6. An isolated GDF-9 nucleic acid molecule as claimed in claim 4, wherein said amino acid substitution is present in a dimerisation domain and disrupts dimerisation.

7. An isolated GDF-9B nucleic acid molecule comprising at least one mutation at position 718, 747 or 1100 of the sequence.

8. An isolated GDF-9B nucleic acid molecule as claimed in claim 7, wherein said mutation results in an amino acid substitution in the polypeptide encoded by said nucleic acid sequence.

9. An isolated GDF-9B nucleic acid molecule as claimed in claim 8, wherein said amino acid substitution is present in a receptor binding domain and disrupts receptor binding.

10. An isolated GDF-9B nucleic acid molecule as claimed in claim 8, wherein said amino acid substitution is present in a dimerisation domain and disrupts dimerisation.

11. A method of identifying a mammal which carries a mutated nucleic acid molecule encoding GDF-9B, said method comprising the steps of:

i) obtaining a tissue or blood sample from the mammal;

ii) isolating DNA from the sample; and optionally

iii) isolating GDF-9B DNA from the DNA obtained at step i) or ii);

iv) probing said DNA with a probe complementary to either strand of the mutated GDF-9B DNA of SEQ ID NOs 11 or 17;

v) amplifying the amount of mutated GDF-9B DNA;